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CARIES INHIBITING AGENT [Ushoku yobo zai]

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Specification

1. Title of the Invention

Caries Inhibiting Agent

2. What is Claimed is:

A caries inhibiting agent comprising either magnolol or honokiol or their mixture.

3. Detailed Description of the Invention

The present invention relates to caries inhibiting agents and, more specifically, to an oral care product that prevents caries or inhibits its progression.

Dental caries, which is generally referred to as cavities, is a disease in which the teeth are limitedly and progressively destroyed; the incidence rate of the disease is significantly high, and it is therefore an important current public health issue.

According to the current research, the cause of dental caries is considered to be sucrose in foods being altered so as to produce insoluble and adhesive glucans (polysaccharide consisting of D-glucose) by an oral Streptococcus. Specifically, due to the glucans produced as described above, bacteria attach themselves to the teeth and grow, forming plaque, which is a source of bacteria. This process is the first step in dental caries, and the bacteria living in the plaque then advance the dental caries by demineralizing the composition of the teeth through acids produced by sugar fermentation.

Since the nature and origin of dental caries are those of an infectious disease, as described above, in order to prevent the dental caries and inhibit their progression, the oral Streptococcus that is the main cause must be eradicated.

Known examples of cariogenic bacteria include Streptococcus mutans, S. sanguis and S. mitis, with S. mutans being known to have the strongest cariogenicity. Specifically, the known key characteristics of the bacterium include adhesion to the surface of the teeth (property requiring sucrose), a bacterial agglutination reaction (high molecular weight dextran-induced) and lactate production by sorbitol and mannitol fermentation. These characteristics significantly contribute to inducing dental caries.

Many attempts have been made to eliminate the oral bacteria for caries prevention; for example, attempts using antibiotics, such as penicillin and erythromycin, cell-wall digesting enzymes and antiseptics, such as chlorhexidine, have actually been made to some extent. However, the above-mentioned substances may be accompanied by side effects, such as disrupting the natural bacterial balance by disturbing oral and intestinal flora; antibiotics in particular significantly exhibit this tendency; thus, none of the above-mentioned substances is widely used. In conclusion, no particular prevention method for dental caries has been proposed, and it is therefore obvious that there is no better caries-preventing method than physical cleaning.

In view of the above-described problems, the present inventors conducted research in order to prevent dental caries and inhibit their progression using oriental medicines, and discovered that a few kinds of oriental medicines have excellent anti-cariogenic bacterial properties as well as the active constituents. The present invention is based on this discovery. The present invention is explained in the following based on the research process and the resulst.

Firstly, the present inventors selected dozens of oriental medicines that had been reported to have antibacterial action, and a sensitivity test of S. mutans was conducted on their methanol, 50% methanol and water extracts using a paper disk method. Type C and type D, which account

for the majority of the Japanese serum group of the 7 serum groups of the bacterium were used as the bacterial test strains. An inhibition circle diameter less than 9 mm for a paper disk diameter 8 mm was taken as (-) and greater than 9 mm as (+), as (+) to (+++++). The evaluation was made using five-grade extract concentrations. Specifically, when 0.1 mg, 0.2 mg, 0.4 mg, 0.8 mg and 1.2 mg of extracts were added to each paper disk, the case in which all the paper disks formed a clear inhibition circle having a diameter of greater than 9 mm was taken as (+++++), and the case in which an inhibition circle was not observed from the paper disk to which 0.1 mg of the extract was added but an inhibition circle was observed from the others was taken as (++++).

Similarly, the case in which an inhibition circle was observed from the paper disks to which greater than 0.4 mg of extract was added was taken as (+++), greater than 0.8 as (++), greater than 1.2 mg as (+), and the case when no inhibition circle was observed with more than 1.2 mg was taken as (-). The favorable results are shown in Table 1. In Table 1, the upper row is the data for type-c and the lower row for type-d.

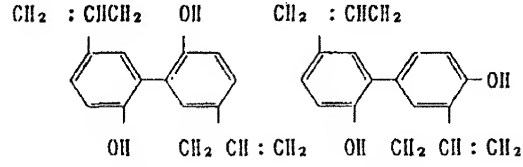
Table 1

	Methanol extract	50% methanol extract	Water extract
Magnolia officinalis	+++++ +++++	+++++ +++++	+++ ++
Magnolia obovata	++++ +++	++++ ++++	+ +
Coptis rhizome	+++++ +++	+++ +++	+++ +++
Japanese gall	++++ +++	+++ ++	++ -
Geranium herb	++++ ++	± +	+ +
Ginkgo leaf	++++ ++	+ -	- -
Bletilla striata	+++ +++	- -	- -
Phellodendron Bark	++ +++	++ ++	++ ++
Gayangae rhizoma	+++ +++	++ -	- -
Pulsatilla root	++ ++	- -	- -
Houttuyniae herba	+++ +++	+++ ++	- -
Prunellae spica	+++ ++	± +	- -
Salvia multiorrhiza root	++ ++	± -	- -
Rhei rhizoma	++ +	+ +	++ -
Gum olibanum	++ -	- -	- -
Anemarrhenae rhizoma	+ +	++ +	- -

Sinomenium stem	+ +	+ -	- -
Scutellaria root	+ +++	- -	- -
Sasa albo-marginata	± ±	± -	+++ ++
Hydrocarpus seed	- +	+ ++	- -
Lithospermi radix	- -	- -	+ +
Clematis radix cum rhizoma	+ +	± -	- -

As shown in the above table, of the tested oriental medicines, magnolia officinalis and magnolia obovata in particular showed excellent results, and in this experiment, methanol extract provided favorable results overall. Bearing in mind the above-described fact, further research was conducted on magnolia bark.

Specifically, when ether extract of magnolia officinalis was fractionated into rough fractions using the usual method and the fractions were subjected to a paper disk method, bactericidal activity against cariogenic bacteria was observed in the acidic fraction. Since magnolia bark is known to contain magnolol (I) and honokiol (II) as phenolic compounds (pharmaceutics magazines 50, 183, 1930 and 93, 422, 1973), both substances were then examined and found to have bactericidal activity against all the 7 serum types of S. mutans.



Magnolol (I) Honokiol (II)

Subsequently, the bactericidal activity against S. mutans was investigated with the magnolia officinalis extracts obtained by other solvents, and various magnolia obovata extracts, and further, the bacterial activity was examined on berberine, an antimicrobial principle of Coptis rhizome, and erythromycin, which showed bactericidal activity against S. mutans, for comparison.

More specifically, methanol, ether and waterextracts of magnolia officinalis, methanol and water extracts of magnolia officinalis and Coptis rhizome, magnolol, honokiol, berberine and erythromycin were selected and their antibacterial activities against the 7 serum types (a to g types) of S. mutans were compared using the paper disk method. Further, the antibacterial activities were evaluated using the largest inhibition circle diameter when 1.2 mg of an extract or 0.006 mg of a composition was added. The results are shown in Table 2.

Table 2

		Concentration (mg/disk)	Largest inhibition circle diameter (mm)						
			Type a	Type b	Type c	Type d	Type e	Type f	Type g
Magnolia obovata	Methanol extract	1.2	13.4	14.4	14.0	11.7	15.6	16.0	12.7
	Ether extract	1.2	15.2	15.3	16.5	13.5	16.1	17.1	13.9
	Water extract	1.2	9.2	-	9.1	8.6	10.1	9.2	9.0
Magnolia officinalis	Methanol extract	1.2	17.8	15.5	18.5	15.8	18.8	21.3	17.4
	Water extract	1.2	12.8	10.0	13.3	10.2	14.3	14.7	13.4
Coptis rhizome	Methanol extract	1.2	16.7	16.3	16.5	17.5	17.9	20.6	16.8
	Water extract	1.2	14.1	11.8	12.7	11.8	14.8	14.2	13.3
Magnolol		0.06	18.1	16.0	16.5	17.5	17.9	20.6	16.8
Honokiol		0.06	18.5	15.1	20.4	16.4	20.0	20.9	17.9
Berberine		0.06	10.1	10.9	9.9	9.6	9.6	9.8	10.5
Erythromycin		0.06	36.3	37.4	36.5	39.2	40.5	36.4	37.4

The results shown in Tables 1 and 2 suggest that magnolia bark extract (including magnolia obovata and magnolia officinalis) and their constituents, such as magnolol and honokiol, significantly inhibit the growth of the cariogenic S. mutans. Therefore, they are very useful as a caries inhibiting agent and more specifically, an oral care product that prevents caries or inhibits its progression. Further, the minimum inhibitory concentrations for magnolia obovata, magnolia officinalis and magnolol are 100 μg/mL, 25μg/mL and 6.25 μg/mL, respectively. The minimum inhibitory concentration for honokiol is 6.25μg/mL.

This means that S. mutans can be killed with:

magnolia obovata having a concentration of 0.01%,

magnolia officinalis having a concentration of 0.0025%,

magnolol having a concentration of 0.000625% and

honokiol having a concentration of 0.000625%.

Although the activity is slightly weaker than prior art erythromycin, erythromycin is an antibiotic and it is therefore difficult to be administered for a long period due to its side effects (ie, disrupting the natural bacterial balance and appearance of resistant bacteria) while the inventive caries inhibiting agent is a crude drugs preparation or a crude drug-origin substance and has less side effects, resulting in clinical concern being significantly reduced. Since caries inhibiting agents are naturally administered for a long period consistently or intermittently, the inventive caries inhibiting agent that has lower concern of side effects is expected to be very useful in practical use. In particular, since oriental medicines that can be obtained by bark extract have been used as an internal agent, side effects caused by their crude drug extract are normally not experienced.

Further, the inventive caries inhibiting agent shows bactericidal activity against cariogenic bacteria with a low concentration in a short time, which is also an excellent aspect of the present invention. This aspect is described below:

For example, the minimal inhibitory concentration of berberine against S. mutans that was

determined using a broth dilution method was as low as 67 μg/mL. Further, both magnolol and honokiol having a low concentration of 7 μg/mL were found to inhibit the growth of S. mutans. Further, magnolol and honokiol were exposed to S. mutans (type-c strain) at a concentration of 70 μg/mL in order to examine the duration of activity and the antibacterial effect; the antibacterial activity of both compounds was found to be bactericidal and to immediately exhibit bactericidal activity within a 2-minute contact time, and a 10-minute contact time was found to be sufficient to fully kill all the bacteria.

The fact that the inventive caries inhibiting agent described above exhibits such a remarkable effect at a low concentration and in a short time indicates that the present invention has many practical uses.

Further, some of the crude drugs and their extracts and compounds are already known to have antibacterial effects.

However, the antibacterial activities that have been reported are for germs, such as coliform bacillus, dysentery bacillus, tubercle bacillus and Staphylococcus aureus, and have not yet been reported in relation to antibacterial activity for S. mutans, the main cause of dental caries. In addition, since S. mutans is so unique that it is not classified in Lancefield's taxonomy of Streptococcus, the present invention is a novel, useful and progressive invention that conventional knowledge is not able to foresee and predict.

The inventive caries inhibiting agent can be used alone or in a mixture.

The inventive caries inhibiting agent can be applied directly in the mouth as it is and can also be mixed with other oral care products, such as toothpaste. As appropriate, the inventive caries inhibiting agent can also be transformed into desired forms, such as lozenges and sublingual tablets.

An appropriate dose should be administered according to the results in Tables 1 and 2, but a slight excess quantity is preferably used considering the amount of loss during application (a fair amount of the dose may be rinsed away with toothpaste). As mentioned above, the inventive caries inhibiting agent has few side effects and can therefore be free of any adverse affects caused by an excessive dose.

The following embodiments illustrate the present invention; needless to say, they are just examples and the present invention is therefore not restricted by them.

Embodiment 1

A product was prepared by cold-extracting crushed Magnolia obovata with ether, fractionating the resulting ether extract into an acidic fraction, a neutral fraction and an alkaline fraction, and collecting the acidic fraction.

Embodiment 2

A mouth wash was prepared by applying column chromatography to the acidic fraction in Embodiment 1 in order to isolate magnolol and honokiol, dissolving them in a small amount of alcohol and adding water and a solubilizing agent.

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